

-1-

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IONENE POLYMERS AND THEIR USE IN TREATING MUCOSITIS

RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No.
5 60/262,586, filed on January 18, 2001. The entire teachings of the above application are
incorporated herein by reference.

BACKGROUND OF THE INVENTION

Oral mucositis is a common, painful, dose-limiting toxicity of drug and radiation
10 therapy for cancer. The disorder is characterized by breakdown of the oral mucosa,
which results in the formation of ulcerative lesions. In granulocytopenic patients, the
ulcerations that accompany mucositis are frequent portals of entry for indigenous oral
bacteria leading to sepsis or bacteremia. Mucositis occurs to some degree in more than
one third of all patients receiving anti-neoplastic drug therapy, and there are about one
15 million occurrences of oral mucositis annually in the United States. The frequency and
severity are significantly greater among patients who are treated with induction therapy
for leukemia or with many of the conditioning regimens for bone marrow transplant.
Among these individuals, moderate to severe mucositis (ulceration) is not unusual in
more than three-quarters of patients. The incidence of mucositis is even higher in
20 younger patients. Moderate to severe mucositis occurs in virtually all patients who



receive radiation therapy for tumors of the head and neck and typically begins with cumulative exposures of 15 Gy and then worsens as total doses of 60 Gy or more are reached.

Clinically mucositis progresses through four stages: (1) An initial stage that is characterized by inflammatory changes of erythema and edema. Localized islands of hyperkeratosis may also be seen. This stage is symptomatically mild and may be successfully palliated by topical anesthetics. (2) Subsequently the mucosa breaks down and becomes eroded and atrophic with increasingly significant inflammatory changes. This stage is increasingly painful and may require systemic analgesic therapy in the form of NSAIDs or oral narcotics for adequate palliation. (3) The third stage of mucositis is the most symptomatic. Full thickness ulcers of the mucosa cause severe discomfort necessitating parenteral narcotic therapy. In addition, in the myelosuppressive patient, these ulcerations provide a systemic portal of entry for the oral microflora often leading to bacteremia and sepsis. Antimicrobial intervention is required. (4) Finally, spontaneous healing occurs 2-3 weeks after cessation of anti-neoplastic therapy.

The complexity of mucositis as a biological process has only been recently appreciated. The condition appears to represent a sequential interaction of oral mucosal cells and tissues including connective tissue, endothelium, epithelium, and inflammatory cells, pro-inflammatory cytokines and local environmental factors such as bacteria and saliva. Damage to epithelial and connective tissue induces release of inflammatory cytokines leading to mucosal damage. Additionally, both direct and indirect effects to epithelial cells result in either apoptotic or necrotic changes in the basal layer; differentiation into new epithelial cells is halted. The arrest of epithelial cell renewal leads to atrophy followed by ulceration.

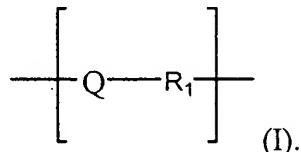
Standard therapy for mucositis is predominantly palliative, including application of topical analgesics such as lidocaine and/or systemic administration of narcotics and antibiotics. No standard curative treatment for mucositis exists. Chlorhexidine mouthwash is extensively used in oral mucositis treatment and prevention, however, its efficacy is decreased in saliva and it is relatively ineffective against the Gram negative

bacteria that tend to colonize the oral cavity. Thus, there is a need for new treatments that inhibit, prevent, reduce the severity, and/or promote the healing of mucositis.

SUMMARY OF THE INVENTION

5 This invention relates to the use of polyionenes are effective in treating or preventing oral mucositis in hamsters. For example, the polyionene poly(4,4'-trimethylenebis(1-methylpiperidinium)-alt-octane (X) was effective in significantly reducing the severity of oral mucositis at a concentration as low as 1.0 mg/mL. This contrasts with chlorhexidine, which is commonly used to treat oral mucositis but was
10 unsuccessful in treating the hamster model at a concentration of 0.5% (v/v). Based on this discovery, methods of treating and/or preventing mucositis in a mammal are disclosed.

The method of treating mucositis comprises administering to the mammal an effective amount of an ionene polymer. In a preferred embodiment of the present
15 invention, the ionene polymer comprises a repeat unit represented by Structural Formula (I):

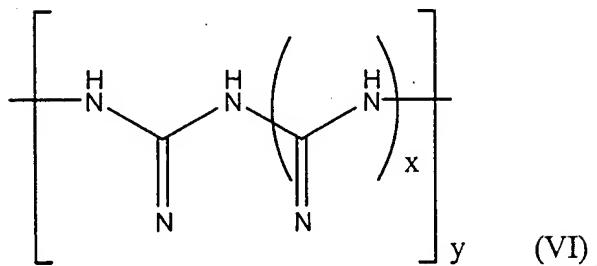
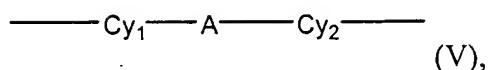
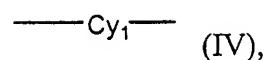
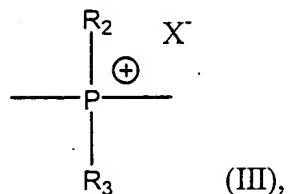
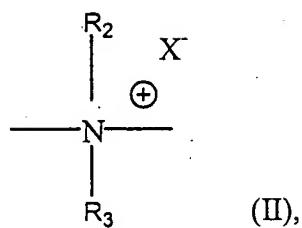


The polymer may be comprised of identical or non-identical repeat units so as to form either a homopolymer or a copolymer.

20 R_1 is a substituted or unsubstituted hydrocarbyl group. Preferably, R_1 is a substituted or unsubstituted arylene or lower alkylene group.

Each Q is represented by Structural Formula (II), (III), (IV), (V), or (VI):

-4-



Cy_1 and Cy_2 are each independently a quaternary nitrogen-containing monocyclic heteroaromatic ring or non-aromatic heterocyclic ring.

A is a covalent bond, or a substituted or unsubstituted lower alkylene group.

5 R₂ and R₃ are independently a substituted or unsubstituted aliphatic or aromatic group. Preferably, in the repeat units of formulae (II) and (III), R₂ and R₃ are each independently an alkyl group or a hydroxyalkyl group.

Each X^- , separately or taken together with other X^- 's, is a physiologically acceptable anion.

The values x and y are integers, where x is an integer from 0-4 or from 1-4 and y is an integer from 1-5 or from 2-5.

The ionene polymers of the present invention have been found to be effective in the treatment of oral mucositis. The ionene polymers of this invention additionally have
5 been found to be non-irritating and low in toxicity to warm-blooded animals.

DETAILED DESCRIPTION OF THE INVENTION

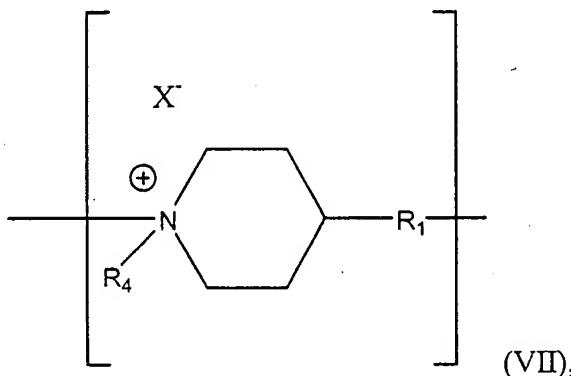
The present invention provides a method of using ionene polymers in pharmaceutical compositions for the treatment of mucositis. "Ionene polymers" or
10 "polyionenes," as used in the present invention, are cationic polymers or copolymers with quaternized nitrogen or phosphorus located in the main polymeric chain or backbone of the polymer, providing a positive charge. Polyionenes can also be polyguanidines or copolymers thereof, where the cationic nitrogen atom is an imide nitrogen directly bonded to the polymer backbone. The molecular weight of the ionene
15 polymers of the present invention is generally not limiting, but each polymer typically comprises from 50 to about 500 repeat units.

Mucositis is defined herein as inflammation and/or ulceration of a mucous membrane. The disclosed method can be used to treat mucositis in the stomach, intestines, and the like; however, it is particularly effective when used to treat oral
20 mucositis. Oral mucositis is characterized by inflammation of a mucous membrane of the oral cavity or lips and is typically accompanied by redness, swelling, and/or ulcerations of the mouth. Included in this description is oral mucositis that is a side-effect of anti-cancer therapies such as chemotherapy and radiotherapy, and oral mucositis that is a side effect of bone marrow transplantation or stem cell transplant or
25 ablation. Mucositis also includes mucositis that develops spontaneously in a healthy patient not receiving anti-cancer therapy, as in the case of a canker sore or mouth ulcer.

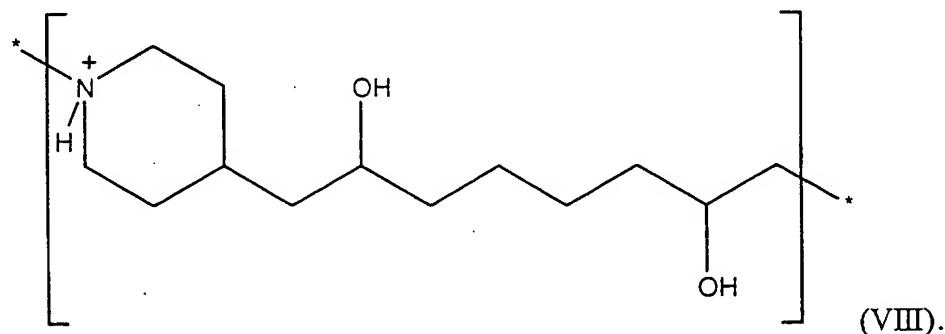
Treatment includes both prophylactic and therapeutic uses of the ionene polymers. Desired prophylactic effects include prevention of and inhibition of mucositis, reduction in severity of mucositis, reduction in size of mucositis lesions
30 compared with, for example, what is normally experienced by a mammal undergoing

cancer therapy, and reduction in likelihood of developing mucositis. Desired therapeutic effects include amelioration of the discomfort associated with the oral mucositis, and/or increased rate of healing of mucositis lesions compared with, for example, what is normally experienced by a mammal undergoing cancer therapy. Thus, 5 the invention provides, in one aspect, a method of treating mucositis or oral mucositis comprising administering an effective amount of an ionene polymer.

In a preferred embodiment of the present invention, Q is represented by Structural Formula (IV) and Cy₁ is a piperidinium ring having a quaternary nitrogen additionally substituted with a hydrogen or a substituted or unsubstituted lower alkyl group. More preferably, the quaternary nitrogen is additionally substituted with a lower 10 alkyl or hydroxy substituted lower alkyl group. An example of a "piperidinium" ionene repeat unit is represented in Structural Formula (VII):

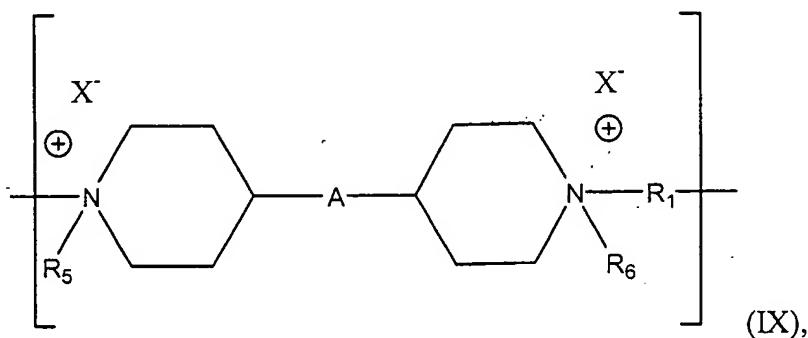


where R₄ is hydrogen or a substituted or unsubstituted lower alkyl group and R₁ is as 15 defined above. A specific example of a piperidinium ionene repeat unit is shown in Structural Formula (VIII):



In another preferred embodiment, Q is represented by Structural Formula (V) and Cy₁ and Cy₂ are each piperidinium rings having a quaternary nitrogen additionally substituted independently with a hydrogen or a substituted or unsubstituted lower alkyl group and A is as defined above. More preferably, the quaternary nitrogen is

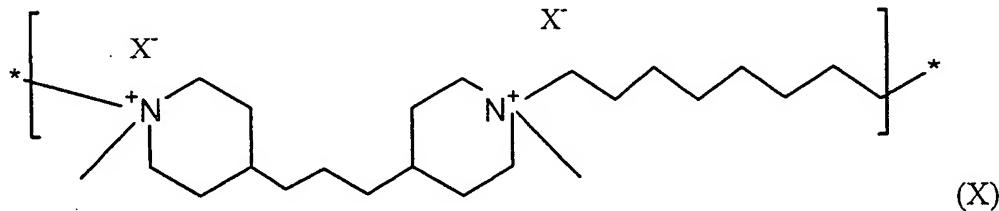
5 additionally substituted with a lower alkyl or hydroxy substituted lower alkyl group. An example of a "piperidinium" ionene repeat unit of this type is represented in Structural Formula (IX):

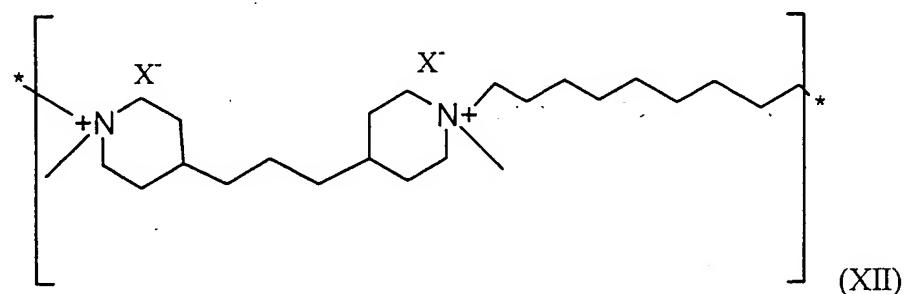
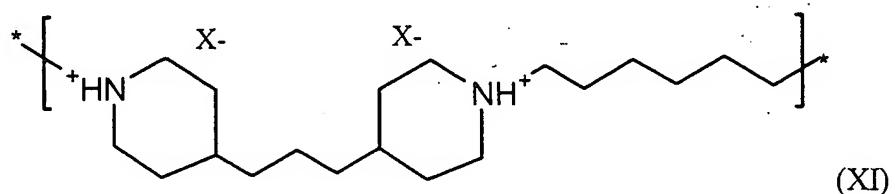


10 where A and R₁ are as defined above, and R₅ and R₆ are each independently hydrogen or a substituted or unsubstituted lower alkyl group. Preferably, R₅ and R₆ are each independently an alkyl group or a hydroxyalkyl group, and A is an unsubstituted straight chained lower alkylene group. Even more preferably, A is an unsubstituted straight chained lower alkylene group and R₁ is a substituted or unsubstituted straight chained lower alkylene or polyalkylene group optionally substituted with one or more hydroxyl groups, preferably an unsubstituted polyalkylene glycol or

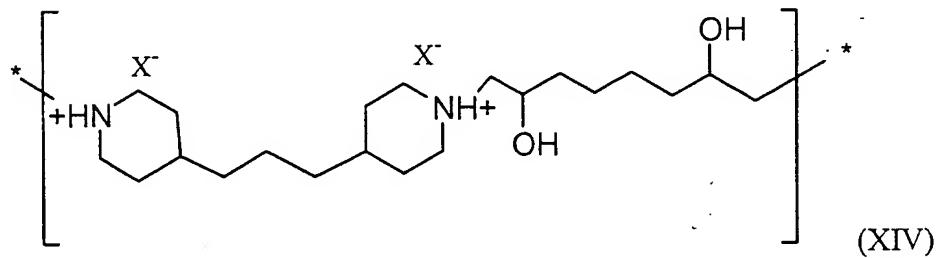
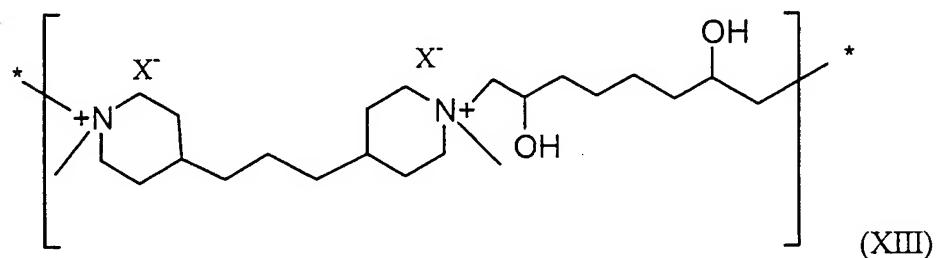
15 -CH₂CHOH(CH₂)_nCHOHCH₂- where n is an integer ranging from 0 to 8. Specific examples of "piperidinium" ionene repeat units are represented by the Structural Formulas (X), (XI), (XII), (XIII), (XIV), and (XV):

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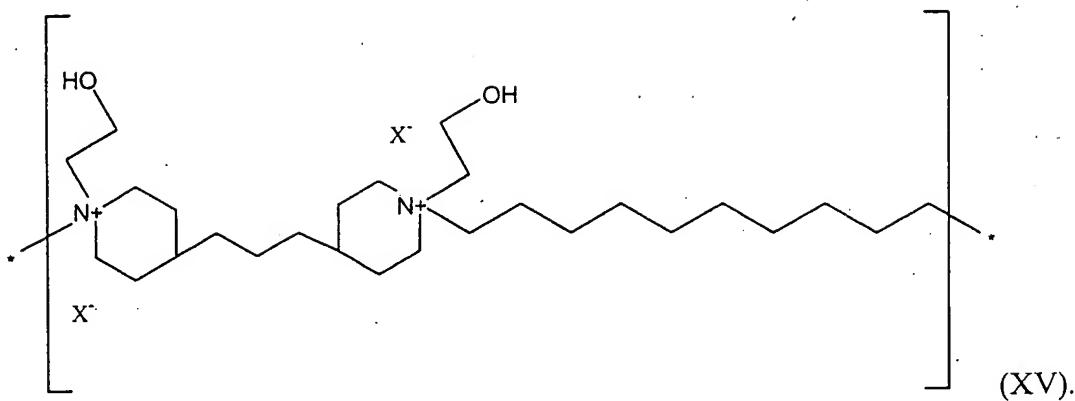




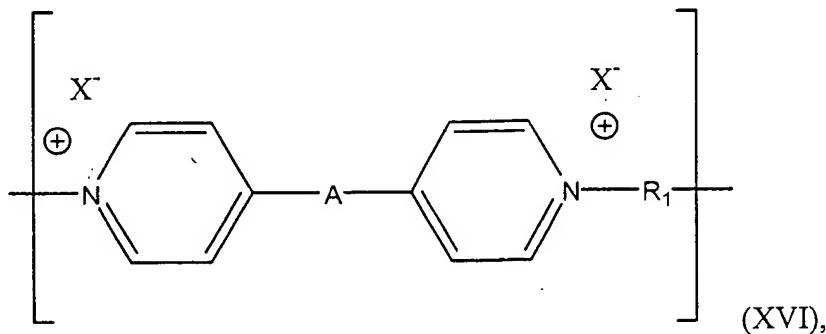
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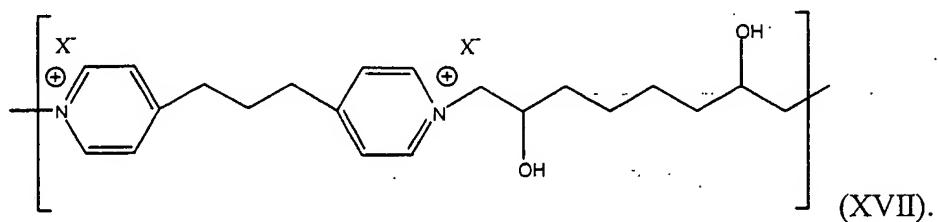
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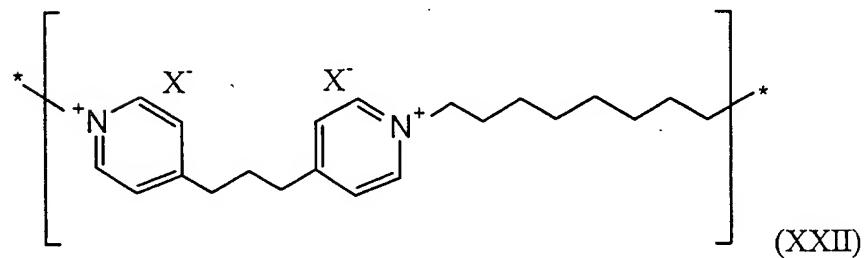
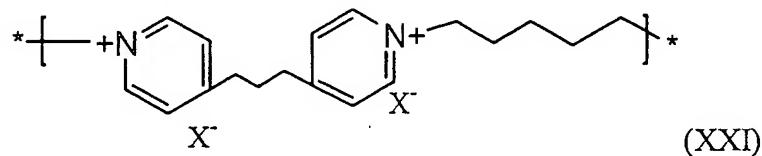
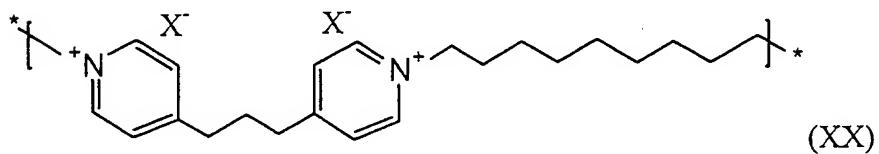
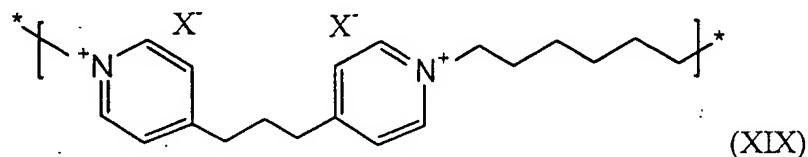
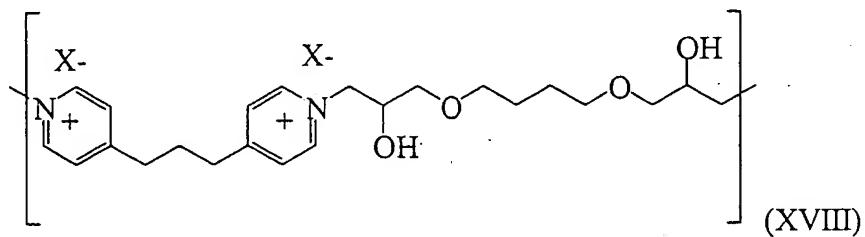
In yet another preferred embodiment, Q is represented by Structural Formula (V) and Cy₁ and Cy₂ are each pyridinium groups and A is as defined above. In one example 5 of a "pyridinium" ionene polymer of this type, the polymer is characterized by repeat units represented by Structural Formula (XVI):

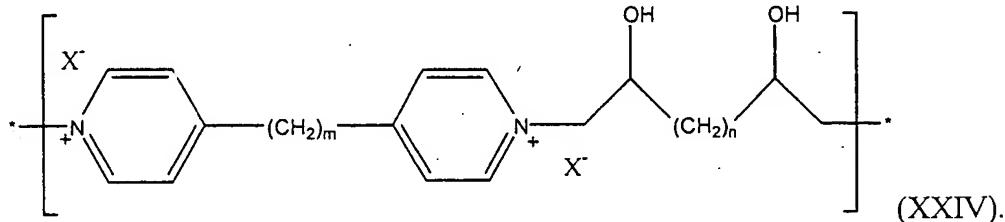
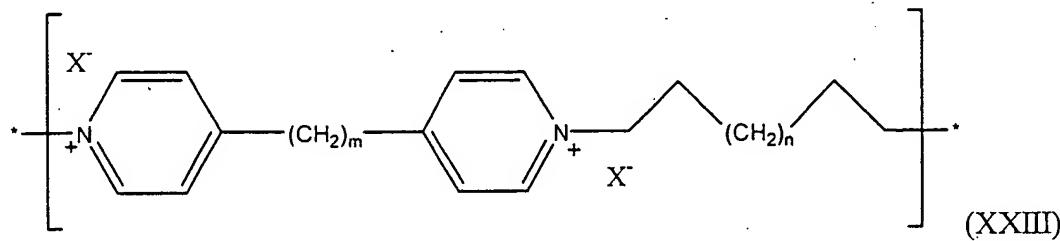


in which A and R₁ are as defined above. In a more preferred embodiment, A is an unsubstituted straight chained lower alkylene group. Even more preferably, A is an unsubstituted straight chained lower alkylene group and R₁ is a substituted or unsubstituted straight chained lower alkylene or polyalkylene glycol group optionally substituted with one or more hydroxyl groups, preferably an unsubstituted polyalkylene or -CH₂CHOH(CH₂)_nCHOHCH₂- where n is an integer ranging from 0 to 8. An 10 example of a repeat unit with these components is represented by Structural Formula 15 (XVII):



Other specific examples of "pyridinium" ionene polymers are represented by Structural Formulas (XVIII), (XIX), (XX), (XXI), (XXII), (XXIII), and (XXIV):





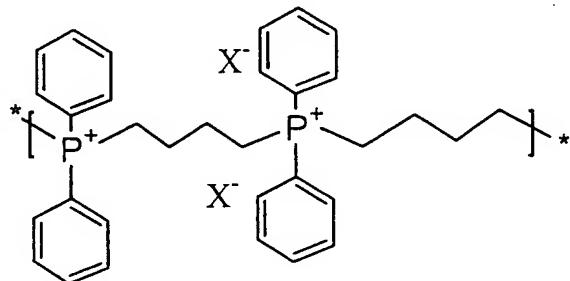
Other specific examples of repeat units of polyionenes that can be used in the disclosed method are represented by Structural Formula (XXIII) above, wherein m=1
5 and n=0; m=1 and n=1; m=1 and n=2; m=1 and n=4; m=1 and n=5; m=1 and n=6; m=1 and n=8; m=2 and n=0; m=2 and n=1; m=2 and n=2; m=2 and n=4; m=2 and n=5; m=2 and n=6; m=2 and n=8; m=3 and n=0; m=3 and n=1; m=3 and n=2; m=3 and n=4; m=3 and n=5; m=3 and n=6; m=3 and n=8; m=4 and n=0; m=4 and n=1; m=4 and n=2; m=4 and n=4; m=4 and n=5; m=4 and n=6; m=4 and n=8; m=5 and n=0; m=5 and n=1; m=5 and n=2; m=5 and n=4; m=5 and n=5; m=5 and n=6; and m=5 and n=8.

Other specific examples of repeat units of polyionenes that can be used in the disclosed method are represented by Structural Formula (XXIV) above, wherein m=1 and n=0; m=1 and n=1; m=1 and n=2; m=1 and n=4; m=1 and n=5; m=1 and n=6; m=1 and n=8; m=2 and n=0; m=2 and n=1; m=2 and n=2; m=2 and n=4; m=2 and n=5; m=2 and n=6; m=2 and n=8; m=3 and n=0; m=3 and n=1; m=3 and n=2; m=3 and n=4; m=3 and n=5; m=3 and n=6; m=3 and n=8; m=4 and n=0; m=4 and n=1; m=4 and n=2; m=4 and n=4; m=4 and n=5; m=4 and n=6; m=4 and n=8; m=5 and n=0; m=5 and n=1; m=5 and n=2; m=5 and n=4; m=5 and n=5; m=5 and n=6; and m=5 and n=8.

Another polyionene suitable for use in the present invention comprises a repeat unit where Q is represented by Structural Formula (II). When Q is represented by Structural Formula (II), R₁ is preferably a substituted or unsubstituted phenylene, lower alkylene, polyalkylene glycol group, or -CH₂CHOH(CH₂)_nCHOHCH₂- where n is an integer ranging from 0 to 8, and R₂ and R₃ are as defined above. Even more preferably,

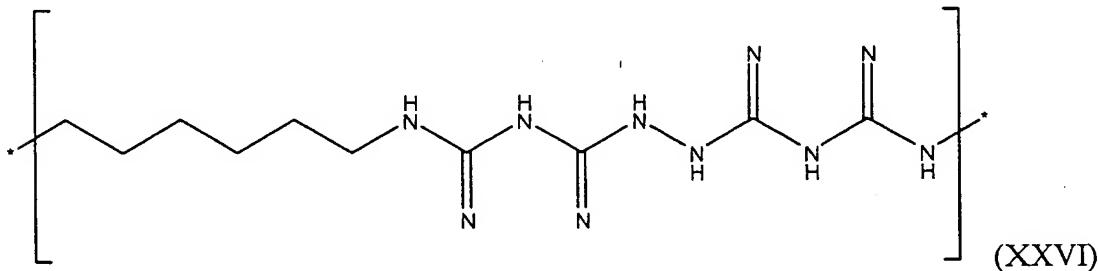
R_1 is a substituted or unsubstituted straight chained lower alkylene group or polyalkylene glycol optionally substituted with one or more hydroxyl groups.

Yet another polyionene suitable for use in the present invention comprises a repeat unit where Q is represented by Structural Formula (III). When Q is represented by Structural Formula (III), R_1 is preferably a substituted or unsubstituted arylene, lower alkylene, polalkylene glycol group, or $-\text{CH}_2\text{CHOH}(\text{CH}_2)_n\text{CHOHCH}_2-$, where n is integer ranging from 0 to 8, and R_2 and R_3 are as defined above. Even more preferably, R_1 is a substituted or unsubstituted straight chained lower alkylene group or polyalkylene glycol optionally substituted with one or more hydroxyl groups. A specific example is represented by Structural Formula (XXV):



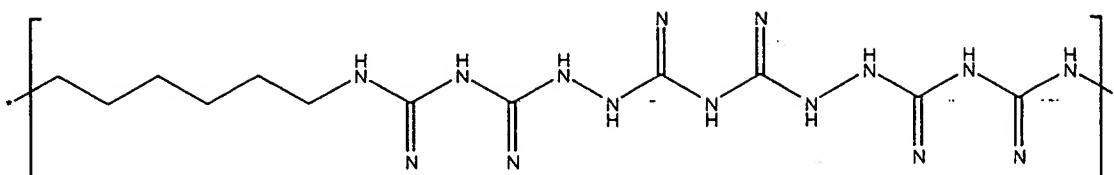
(XXV).

In another embodiment of the present invention, Q is represented by Structural Formula (VI). Preferably, R_1 is an unsubstituted lower alkylene or lower alkylene glycol group and x is 1 and y is 2; x is 1 and y is 3; x is 1 and y is 4; or x is 1 and y is 5. Specific examples of guanidine ionene polymers and copolymers comprise repeat units of formulas (XXVI), (XXVII), (XXVIII), and (XXIX):

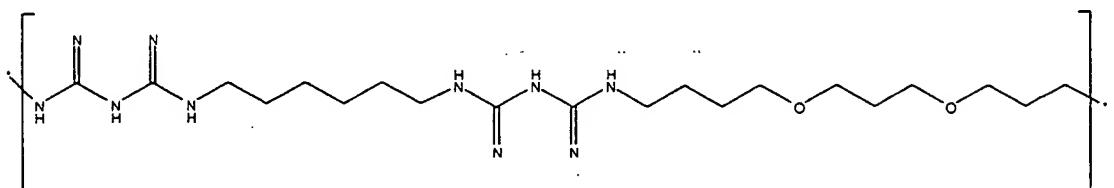


(XXVI)

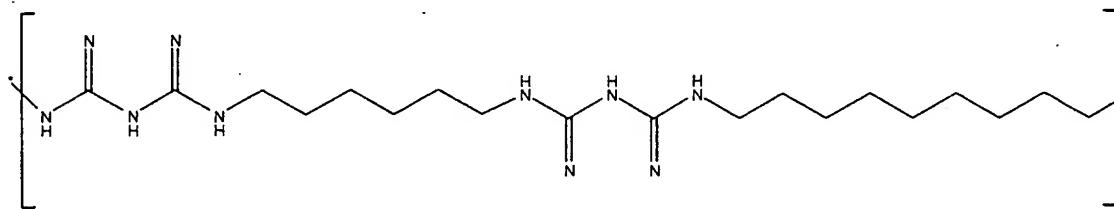
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(XXVII)



(XXVIII)

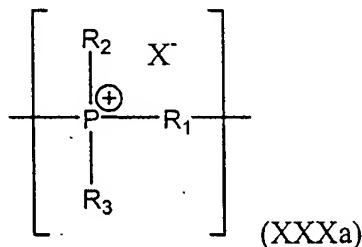


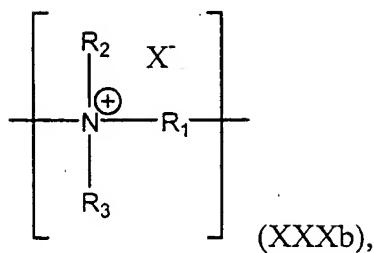
(XXIX)

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As noted above, ionene polymers suitable for use in the disclosed method include homopolymers and copolymers. The variables in each repeat unit of a copolymer of the present invention are independently selected. For example, in a 10 copolymer, the alkylene group represented by A in one repeat unit can differ from the alkylene group represented by A in other repeat units. Alternatively, Q is identical in all repeat units and R₁ varies; R₁ is identical in all repeat units and Q varies; or Q and R₁ each vary among repeat units. In a homopolymer Q, R₁, and A are identical in all repeat units.

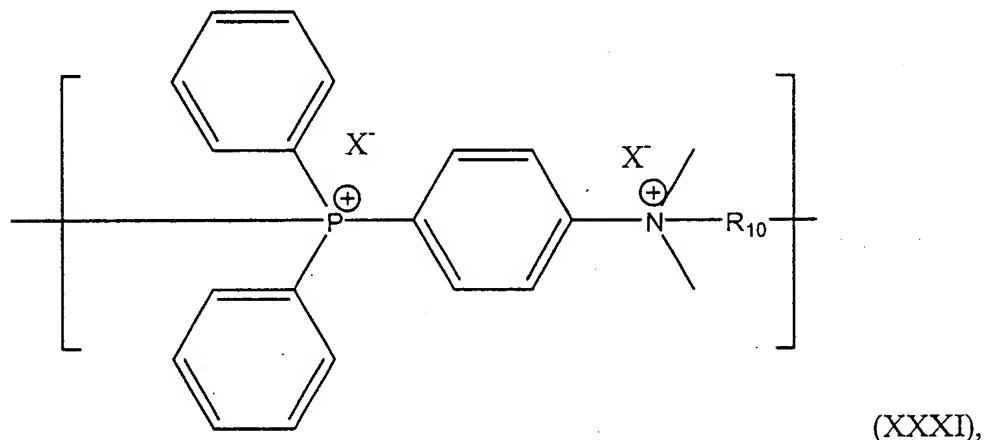
15 In one example of an ionene copolymer where Q varies within the polymer, Q is represented by Structural Formula (II) and Structural Formula (III). This copolymer is comprised of repeat units represented by Structural Formulas (XXXa) and (XXXb):





where R_1 , R_2 , R_3 and X^- are as defined above, and are chosen independently for each repeat unit. That is, R_1 , R_2 , R_3 , and X^- are not necessarily the same throughout the 5 copolymer.

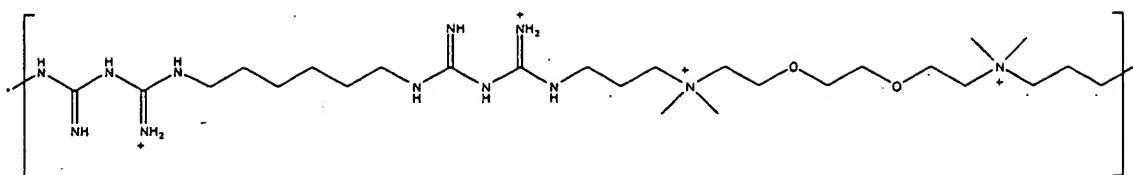
In one example of an ionene copolymer of this type, the repeat units of Structural Formulae (XXXa) and (XXXb) alternate to form a repeat unit represented by Structural Formula (XXXI):



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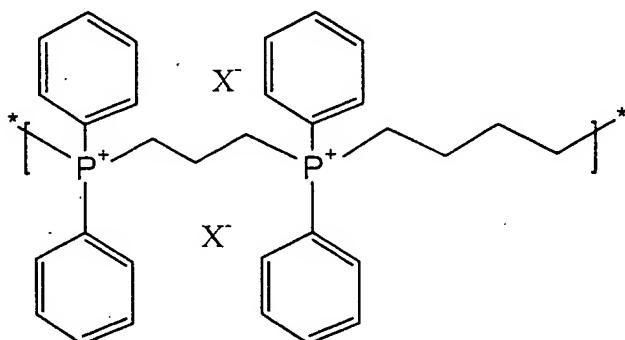
where R_{10} is a substituted or unsubstituted lower alkylene group having 1 to about 24 carbon atoms, preferably having about 4 to about 12 carbon atoms. Each X^- , separately or taken together with other X^- 's, is a physiologically acceptable anion.

In another example of an ionene copolymer where Q varies within the 15 copolymer, Q alternates between repeat units represented by Structural Formulae (II)-(V), (X)-(XV), or (XVII)-(XXII) and a repeat unit represented by Structural Formula (VI). One copolymer of this type is represented by Structural Formula (XXXII):



(XXXII).

One example of a repeat unit of an ionene copolymer where Q is identical and R₁ varies is represented by Structural Formula (XXXIII):



(XXXIII).

5

An "aliphatic group" is non-aromatic, consists solely of carbon and hydrogen and may optionally contain one or more units of unsaturation, e.g., double and/or triple bonds. An aliphatic group may be straight chained, branched, or cyclic and typically contains between about 1 and about 24 carbon atoms, more typically between about 1 and about 12 carbon atoms.

10 Aliphatic groups are preferably lower alkyl groups or lower alkylene groups, which include C1-24 (preferably C1-C12) straight chained or branched saturated hydrocarbons. An alkyl group is a saturated hydrocarbon in a molecule that is bonded to one other group in the molecule through a single covalent bond from one of its carbon atoms. Examples of lower alkyl groups include methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl and *tert*-butyl. An oxyalkyl group is an alkyl group where an oxygen atom connects the alkyl group and one other group. An alkylene group is a saturated hydrocarbon in a molecule that is bonded to two other groups in the molecule through single covalent bonds from two of its carbon atoms. Examples of lower alkylene groups 15 include methylene, ethylene, propylene, *iso*-propylene (-CH(CH₃)CH₂-), butylene, *sec*-butylene (-CH(CH₃)CH₂CH₂-), and *tert*-butylene (-C(CH₃)₂CH₂-). 20

Aromatic groups include carbocyclic aromatic groups such as phenyl, 1-naphthyl, 2-naphthyl, 1-anthracyl and 2-anthacyl, and heterocyclic aromatic groups such as *N*-imidazolyl, 2-imidazole, 2-thienyl, 3-thienyl, 2-furanyl, 3-furanyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 2-pyranyl, 3-pyranyl, 3-pyrazolyl, 4-pyrazolyl, 5-pyrazolyl, 2-pyrazinyl, 2-thiazole, 4-thiazole, 5-thiazole, 2-oxazolyl, 4-oxazolyl and 5-oxazolyl.

Aromatic groups also include fused polycyclic aromatic ring systems in which a carbocyclic aromatic ring or heteroaryl ring is fused to one or more other heteroaryl rings. Examples include 2-benzothienyl, 3-benzothienyl, 2-benzofuranyl, 3-benzofuranyl, 2-indolyl, 3-indolyl, 2-quinolinyl, 3-quinolinyl, 2-benzothiazole, 2-benzooxazole, 2-benzimidazole, 2-quinolinyl, 3-quinolinyl, 1-isoquinolinyl, 3-quinolinyl, 1-isoindolyl and 3-isoindolyl.

Phenyl is a preferred aromatic group.

"Arylene" is an aromatic ring(s) moiety in a molecule that is bonded to two other groups in the molecule through single covalent bonds from two of its ring atoms. Examples include phenylene $[-(C_6H_4)-]$, thienylene $[-(C_4H_2S)-]$ and furanylene $[-(C_4H_2O)-]$.

A polyalkylene glycol is an alkylene group, which includes one or more ether linkages, where the chain includes a total of about 1 to about 12 carbon and oxygen atoms, and is optionally substituted with one or more hydroxyl groups. Preferably, the polyalkylene glycol is polyethylene glycol or polypropylene glycol.

A "hydrocarbyl group" is an alkylene or arylene group, i.e., $-(CH_2)_x-$ or $-(CH_2)_xC_6H_4(CH_2)_x-$ where x is a positive integer (e.g., from 1 to about 30), preferably between 6 and about 30, more preferably between about 6 and about 15. The carbon chain of the hydrocarbyl group may be optionally interrupted with any combination of ether (-O-), thioether (-S-), amine $[-N(R^a)-]$ or ammonium $[-N^+(R^aR^b)-]$ linkages. R^a and R^b are independently -H, alkyl, substituted alkyl, phenyl, or substituted phenyl. R^a and R^b can be the same or different, but are preferably the same. Examples of hydrocarbyl groups include butylene, pentylene, hexylene, heptylene, octylene,

nonylene, decylene, dodecylene, 4-oxaoctylene, 4-azaoctylene, 4-thiaoctylene, 3,6-dioxaoctylene, 3,6-diazaoctylene, and 4,9-dioxadodecane.

Suitable substituents on an aliphatic, aromatic or benzyl group are those that do not substantially decrease the mucositis-treating properties of the molecule (e.g., 5 increase the ED₅₀ by more than a factor of ten). Examples of suitable substituents on an aliphatic, aromatic or benzyl group include, for example, halogen (-Br, -Cl, -I and -F) -OR, -CN, -NO₂, -NR₂, -COOR, -CONR₂, -SO_kR (k is 0, 1 or 2) and -NH-C(=NH)-NH₂. Each R is independently -H, an aliphatic group, a substituted aliphatic group, a benzyl group, a substituted benzyl group, an aromatic group or a substituted aromatic 10 group, and preferably -H, a lower alkyl group, a benzylic group or a phenyl group. A substituted benzylic group or aromatic group can also have an aliphatic or substituted aliphatic group as a substituent. A substituted aliphatic group can also have a benzyl, substituted benzyl, aromatic or substituted aromatic group as a substituent. A substituted aliphatic, substituted aromatic or substituted benzyl group can have more 15 than one substituent. A preferred substituent on an aliphatic group is -OH.

The anions represented by X⁻ in the polymer can be the same or different. Each X⁻ in a repeat unit can separately be a monovalent anion, i.e., an anion having a negative charge of one. Alternatively, two or more X⁻s in the same repeat unit or in different repeat units, taken together, can represent an anion having a negative charge of 20 two, three or more. A polymer can comprise anions of different charges. Examples of suitable counteranions include sulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, fumarate, 25 maleate, benzoate, sulfonate, phenylacetate, citrate, lactate, glycolate, tartrate and the like. Bromide and chloride are preferred. One anion can be exchanged for another by passing a solution containing the desired counter anion over the polymer.

Also included in the present invention are physiologically acceptable salts of the polymers having repeat units represented by Formulas VI and XXVI-XXIX. Salts can

be formed by reacting the polymer with a suitable acid. Examples include the corresponding acid of the salts listed in the previous paragraph. The hydrochloride and hydrobromide salts are preferred. Polymers represented by Formulas VI and XXVI-XXIX can have up to one molecule of hydrochloride or hydrobromide for every

5 -NHC(=NH)NH- group in the repeat unit.

The polymer can be administered alone or in a pharmaceutical composition comprising the polymer, a pharmaceutically acceptable carrier, and optionally, one or more additional drugs, e.g., antibiotics or antimicrobials. Examples include streptomycin, rifamycin, amphotericin B, griseofulvin, penicillin, cephalothin, cefazolin, 10 chloramphenicol, fluconazole, clindamycin, erythromycin, bacitracin, vancomycin, ciprofloxacin, tetracycline, and fusidic acid.

The polymers can be administered, for example, topically, orally, intranasally, by aerosol or rectally. The form in which the polymer is administered, for example, powder, tablet, capsule, solution, or emulsion, depends in part on the route by which it 15 is administered. For oral mucositis, the polymer is preferably administered orally as a gargle, an ointment, a swab, a gel, and the like.

Suitable carriers and diluents for an ionene polymer will be immediately apparent to persons skilled in the art. These carrier and diluent materials, either organic or inorganic in nature, include, for example, gelatin, lactose, starch, magnesium stearate, 20 preservatives (stabilizers), sugars, emulsifying agents, salts and buffers. When applied directly to the lesion, examples of pharmaceutically acceptable carriers include, for example, commercially available inert gels, or liquids supplemented with albumin, methyl cellulose, or a collagen matrix.

An effective amount of an ionene polymer to be administered will be determined 25 on an individual basis, and will be determined at least in part, by consideration of the individual's size, the severity of symptoms to be treated and the result sought. As used herein, an effective amount refers to an appropriate amount of ionene polymer, which results in a desired therapeutic or prophylactic effect with respect to mucositis, as defined above. Typical dosages for applied and/or ingested ionene polymers range from 30 between about 0.05 µg/kg body weight to about 500 mg/kg body weight, more typically

between about 0.1 µg/kg body weight to about 100 mg/kg body weight and even more typically between about 0.5 µg/kg body weight and about 10 mg/kg body weight.

The method of the claimed invention is particularly useful in the treatment of oral mucositis resulting from anti-cancer therapy, such as radiation therapy or

5 chemotherapy, including induction therapy in leukemia patients. The treatment can be particularly beneficial for patients undergoing treatment for tumors of the head and neck, such as radiation patients. For prophylactic treatment of mucositis resulting from chemotherapy, treatment with an ionene polymer is initiated before the onset of the chemotherapy, during chemotherapy, after chemotherapy is complete but before

10 symptoms appear or any combination of the above. For prophylactic treatment of mucositis resulting from radiation therapy, treatment with the ionene polymer is initiated before the onset of radiation therapy, during radiation exposure, after radiation exposure has been terminated (preferably no sooner than about one hour, more preferably five hours after termination) but before symptoms appear or any combination

15 of the above. For therapeutic treatment of mucositis resulting from radiation therapy or chemotherapy, the ionene polymer is administered after symptoms of mucositis (e.g., mouth ulcers) have appeared.

The method is preferably used with human patients, but can also be used with other mammals, such as companion animals (e.g., dogs, cats, and the like), farm animals

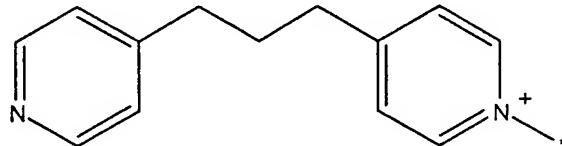
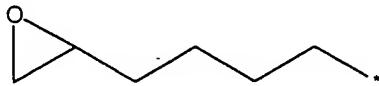
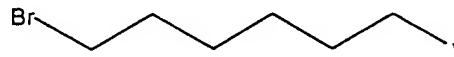
20 (horses, cattle, goats, and the like) and laboratory animals (hamsters, mice, rats, and the like).

Ionene polymers of the present invention can be prepared by reacting a divalent electrophile such as an α,ω -dihalogenated alkane or a corresponding diepoxyde with a divalent nucleophile such as 4,4'-trimethylenedipiperidine or N,N,N',N'-

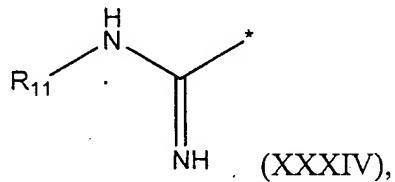
25 tetramethyl-1,3-propanediamine. When preparing a polyguanidine, the divalent nucleophile is an α,ω -diaminoalkane or an α,ω -aminoguanidine and the divalent electrophile typically is an α,ω -biscyanoguanidine. Polymerizing with one divalent electrophile and one divalent nucleophile results in a homopolymer. Polymerizing with two or more divalent electrophiles and/or divalent nucleophiles results in a copolymer.

30 Such homopolymers and copolymers are encompassed within the present invention.

Polyionene polymers are typically "capped" at the termini with a partially reacted divalent electrophile or nucleophile or a monovalent electrophile or nucleophile. For example, when polymerizing 4,4'-trimethylenepyridine and 1,6-dibromohexane (or the corresponding epoxide), the resulting polymer is capped at either end with one of the 5 following groups:



Optionally, the capping group can be reacted further, for example, by hydrolyzing the 10 epoxide or reacting the halide or epoxide with a nucleophile. An example of a capping group for polyguanidine polymers or copolymers is represented by Structural Formula (XXXIV):



where R_{11} is a C2-C90 alkyl, C2-C90 oxyalkyl, or aromatic group and the symbol "*" represents the bond connecting the cap to the polymer or copolymer.

Ionene polymers of the invention may also be cross-linked with primary, 15 secondary or other polyfunctional amine using means known in the art. Ionene polymers can be cross-linked by polymerizing in the presence of a multivalent nucleophile (i.e., a compound with three or more nucleophilic groups such as a triamine or tetraamine) or a multivalent electrophile (i.e., a compound with three or more nucleophilic groups such as a trihalide or tetrahalide).

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims. The invention will now be 5 further and specifically described by the following non-limiting Examples.

EXAMPLES

EXAMPLE 1

Preparation of poly(hexamethylenebiscyanoguanidine-alt-4,9-dioxadodecane) (XXVIII).

10 Hexamethylenebiscyanoguanidine (3.99 mmoles, 1.00 g) and 4,9-dioxa-1,12-dodecanediamine (3.99 mmoles, 0.848 ml) were added to a 40 ml vial with a septa-cap followed by 2 equivalents of concentrated HCl. The mixture was heated to 135-145° C in a shaker overnight. The resulting clear yellow, brittle solid was dissolved in water and purified by centrifugation through a 3K Macrosep filtration membrane.

15

EXAMPLE 2

Preparation of poly(4,4'-trimethylenebis(1-methylpiperidinium)-alt-octane) (X).

4,4'-Trimethylenebis(1-methylpiperidine)-alt-1,8-Dibromoocetane was prepared by dissolving 4,4'-Trimethylenebis(1-methylpiperidine) (39.9 ml) in 30 ml of DMF in a 20 250 ml Erlenmeyer flask. 1,8-Dibromoocetane (27.63 ml) was also added to the flask. The reaction was purged with nitrogen, covered with a septum, and stirred with a magnetic stir plate. The initial solution was clear. After approximately 20 minutes of stirring the reaction exothermed and solidified. A light yellow solid polymer formed and was left to further polymerize for a week. The polymer was dissolved in ~300 ml of 25 deionized water and dialyzed (3500 molecular weight cut-off) in water 3x and 1x in water/MeOH 70%/30%.

EXAMPLE 3

Preparation of poly(4-(dimethylamino)phenyldiphenylphosphonium-alt-dodecane) (XXXI, where R₁₀ is dodecyl).

4-(Dimethylamino)phenyldiphenylphosphine (1.73 mmoles, 0.529 g) and 1,12-dibromododecane (1.73 mmoles, 0.569 g) were dissolved in DMF (1 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

EXAMPLE 4

10 Preparation of poly(4,4'-trimethylenedipyridinium-alt-hexane) (XIX).

4,4'-Trimethylenedipyridine (3.46 mmoles, 0.687 g) was added to a 40 ml vial followed by 2.3 ml of DMF/methanol (1:1 v:v). 1,6-dibromohexane (3.46 mmoles, 0.533 ml) was added and the vial was capped with a septa-cap. The vial was purged with nitrogen and placed in a shaker for 1 week. The resulting clear orange viscous solution was diluted in water and purified by centrifugation through a 3K Macrosep.

EXAMPLE 5

Preparation of poly(4,4'-trimethylenedipyridinium-alt-nonane) (XX).

4,4'-Trimethylenedipyridine (3.46 mmoles, 0.687 g) was added to a 40 ml vial followed by 2.3 ml of DMF/methanol (1:1 v:v). 1,9-dibromononane (3.46 mmoles, 0.705 ml) was added and the vial was capped with a septa-cap. The vial was purged with nitrogen and placed in a shaker for 1 week. The resulting light orange waxy solid was dissolved in water and purified by centrifugation through a 3K Macrosep.

25 EXAMPLE 6

Preparation of poly(N,N-dimethylpropylammonium-alt-N,N-dimethylhexylammonium).

N,N,N',N'-Tetramethyl-1,3-propanediamine-alt-1,6-Dibromohexane was prepared by dissolving N,N,N',N'-Tetramethyl-1,3-propanediamine (31.9 ml) in 40 ml of DMF in a 250 Erlenmeyer flask. 1,6-Dibromohexane (29.3 ml) was added to the

flask. The reaction was purged with nitrogen, covered with a septum, and stirred with a magnetic stir plate. The initial solution was clear. A very quick reaction that exothermed and solidified occurred. An off white solid polymer formed and was left to further polymerize for a week. The polymer was dissolved in approximately 300 ml of deionized water and dialyzed (3500 MW) in water 3x and 1x in water/MeOH 70%/30%.

5

EXAMPLE 7

Preparation of poly(hexamethylene biscyano guanidine -alt-nonane) (XXIX).

Hexamethylenebiscyanoguanidine (3.99 mmoles, 1.00 g) and 1,9-diaminononane (3.99 mmoles, 0.623 g) were added to a 40 ml vial with a septa-cap followed by 2 equivalents of concentrated HCl. The mixture was heated to 135-145° C in a shaker overnight. The solid was dissolved in water and purified by centrifugation through a 3K Macrosep filtration membrane.

15

EXAMPLE 8

Preparation of poly(4,4'-trimethylenedipiperidinium-alt-hexane) (XI).

4,4'-Trimethylenedipiperidine (3.466 mmoles, 1.139 g) was added to a 40 ml vial followed by 2 ml DMF/MeOH (1:1v:v). 1,6-Dibromohexane (3.466 mmoles, 0.533 ml) was added and the vial was capped with a septa-cap. The vial was purged with nitrogen and placed in a shaker for 1 week. The resulting opalescent waxy solid was dissolved in water and purified by centrifugation through a 3K Macrosep.

20

EXAMPLE 9

Preparation of poly(hexamethylenebiscyanoguanidine-alt-hydrazine) (XXVI).

25 Hexamethylene biscyano guanidine (4.00 mmoles, 1.00 g) and hydrazine (4.00 mmoles, 0.274 g) were added to a 40 vial with a septa-cap followed by 2 equivalents of concentrated HCl. The mixture was heated to 165° C in an oil-bath for 3 h. The resulting pink foam was acidified with 2 equivalents concentrated HCl, dissolved in water and purified by centrifugation through a 3K Macrosep filtration membrane.

EXAMPLE 10

Preparation of poly(4-(dimethylamino)phenyldiphenylphosphonium-alt-nonane) (XXXI, where R10 is nonyl).

5 4-(Dimethylamino)phenyldiphenylphosphine (1.73 mmoles, 0.529 g) and 1,9-dibromononane (1.73 mmoles, 0.352 g) were dissolved in DMF (1 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

10 EXAMPLE 11

Preparation of poly(4-(dimethylamino)phenyldiphenylphosphonium-alt-decane) (XXXI, where R10 is decyl).

10 4-(Dimethylamino)phenyldiphenylphosphine (1.73 mmoles, 0.529 g) and 1,10-dibromodecane (1.73 mmoles, 1.04 g) were dissolved in DMF (1 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

EXAMPLE 12

20 Preparation of poly(hexamethylene biscyano guanidine-alt-1,3-aminoguanidine) (XXVII). Hexamethylene biscyano guanidine (4.00 mmoles, 1.00 g) and 1,3-aminoguanidine (4.00 mmoles, 0.502 g) were added to a 40 ml vial with a septa-cap followed by 2 equivalents of concentrated HCl. The mixture was heated to 165° C in an oil-bath for 3 h. The resulting orange solid was acidified with 1 eq. concentrated HCl, dissolved in water and purified by centrifugation through a 3K Macrosep filtration membrane.

EXAMPLE 13

Preparation of poly(1,3-bis(diphenylphosphonium)propane-alt-butane) (XXXIII).

1,3-Bis(diphenylphosphino)propane (1.33 mmoles, 0.550 g) and 1,4-

dibromobutane (1.33 mmoles, 0.159 g) were dissolved in DMF (0.769 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

5 EXAMPLE 14

Preparation of poly(4-(dimethylamino)phenyldiphenylphosphonium-alt-butane) (XXXI, where R10 is butyl).

4-(Dimethylamino)phenyldiphenylphosphine (1.73 mmoles, 0.529 g) and 1,4-dibromobutane (1.73 mmoles, 0.207 g) were dissolved in DMF (1 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

EXAMPLE 15

Preparation of poly(1,4-bis(diphenylphosphonium)butane-alt-butane) (XXV).

15 1,4-Bis(diphenylphosphino)butane (2.31 mmoles, 0.986 g) and 1,4-dibromobutane (2.31 mmoles, 0.276 g) were dissolved in DMF (1.333 ml) and shaken for 1 week. The resulting viscous liquid was diluted with water and purified by centrifugation through a 3K Macrosep.

20 EXAMPLE 16

Preparation of Crosslinked Polymers - Post-polymerization crosslinking

Hydroxyl-containing polymer (XVII) was cross-linked with 6 mole % 1,6-diisocyanatohexane in DMF to produce a gel. The gel was washed with 70% methanol-water and lyophilized.

25

EXAMPLE 17

Preparation of Crosslinked Polymers - *In situ* crosslinking

N,N,N',N'-Tetramethyl-1,3-propanediamine (34.64 mmoles, 5.795 ml), 1,9-dibromononane (34.64 mmoles, 7.048 ml), and

1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene (3.464 mmoles, 1.383 g) were dissolved in DMF (1 ml) and shaken for a week at room temperature. The resulting white gel was washed with hot DMF, methanol, and water and lyophilized.

5

EXAMPLE 18

Preparation of poly(trimethylenedipyridinium-alt-2,7-dihydroxyoctane) (XVI).

Trimethylenedipyridine (100g) was placed in a roundbottom flask. To the flask was added 1,2,7,8-diepoxyoctane (71.72g). The reaction was stirred under nitrogen at 10 room temperature for 20 min. until nearly all the trimethylenedipyridine was dissolved. At this time, acetic acid (121g) was slowly added dropwise over a 24hr period. The reaction was stirred at room temperature for an additional four days. The resulting material was dark blue and highly viscous. The solid was dissolved in water and purified by tangential flow with a 1K MWCO membrane.

15

EXAMPLE 19

Polyionene Polymers Are Effective in Treating Mucositis in a Hamster Model Following Irradiation Therapy

Oral mucositis is a frequent sequel to chemotherapeutic treatment for a number 20 of cancers, as well as of irradiation for head and neck tumors. While the precise causes of mucositis remain unknown, oral microflora are thought to be involved in both the induction and exacerbation of disease. The efficacy of polyionene polymers in treating oral mucositis was assayed according to a hamster model disclosed in Sonis *et al.*, *Oral Oncology* 36:373 (2000), the entire teachings of which are incorporated herein by 25 reference.

Briefly, male Golden Syrian hamsters (Charles River Laboratories), aged 5 to 6 weeks, with body weights of approximately 90 g at project commencement, were used. Mucositis was induced using an acute radiation protocol. A single dose of radiation

(35-40 Gy/dose) was administered to all animals on Day 0. Radiation was generated with a 250 kilovolt potential (15 mA) source at a focal distance of 50 cm, hardened with a 0.35 mm Cu filtration system. Irradiation targeted the left buccal pouch mucosa at a rate of 121.5 cGy/minute. Prior to irradiation, animals were anesthetized with an 5 intraperitoneal injection of sodium pentobarbital (80 mg/kg). The left buccal pouch was everted, fixed and isolated using a lead shield.

All animals were dosed with test material three times per day. A needleless tuberculin syringe containing 0.5 ml of the test compound was inserted into the left cheek pouch and the drug deposited into the pouch. Dosing began on Day 0 and 10 continued until Day 19.

For the evaluation of mucositis, the animals were anesthetized with inhalation anesthetics, and the left pouch everted. Mucositis was scored visually by comparison to a validated photographic scale, ranging from 0 for normal to 5 for severe ulceration . In descriptive terms, this scale is defined as follows:

Score	Description
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray appearance due to a pseudomembrane. Cumulative size of ulcers should equal about 1/4 of the pouch. Severe erythema and vasodilation.
4	Cumulative size of ulcers should equal about 1/2 of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth).

A photograph was taken of each animal's cheek pouch mucosa using a standardized technique. At the conclusion of the experiment, all films were developed and the photographs randomly numbered. At least two independent trained-observers graded the photographs in blinded fashion using the above-described scale (blinded scoring). A score of 1-2 is considered to represent a mild stage of the disease, whereas a score of 3-5 is considered to indicate moderate to severe mucositis in which frank ulceration of the cheek pouch is evident. Treatment efficacy was measured by the reduction in time that the animals experienced ulcerative mucositis (a score < 3) expressed as a percentage of the time that the animals in the control group experienced ulcerative mucositis (a score ≥ 3). Animals treated with polyionene compounds experienced a significant reduction in the percent time they experienced ulcerative mucositis. For example, the chloride salt of an approximately 20 kDa polyionene consisting of repeat units characterized by Structural Formula (X) was effective in treating mucositis, as shown below:

15

Formulation	Concentration (mg/mL)	% Reduction From Control
Chloride Salt	1.0	33.1
Chloride Salt Dissolved in Hydroxypropylmethylcellulose	0.1	33.5
Chloride Salt Dissolved in Hydroxypropylmethylcellulose	1.0	46.6

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed in the scope of the following claims.